Journal of Medical Virology 49:29–33 (1996)

Value of Laboratory Investigations in Clinical Suspicion of Cytomegalovirus-Induced Upper Gastrointestinal Tract Ulcerations in HIV-Infected Patients

J.W. Dorigo-Zetsma, J.T.M. van der Meer, M. Tersmette, F.J.W. ten Kate, P.M.E. Wertheim-van Dillen, and J. van der Noordaa

Laboratory of Medical Microbiology, Department of Clinical Virology (J.W.D.-Z., M.T., P.M.E.W.-v.D., J.v.d.N.), Department of Medicine, Division of Infectious Diseases, Tropical Medicine and AIDS (J.T.M.v.d.M.), and Laboratory of Pathology (F.J.W.t.K.), Academic Medical Center, Amsterdam, The Netherlands

To assess the value of laboratory investigations for the diagnosis and treatment of cytomegalovirus-induced upper gastrointestinal tract ulcerations, the medical records and biopsy material from HIV-infected patients were reviewed retrospectively during a 12-month period. Clinical diagnosis of cytomegalovirus (CMV) ulceration, based on characteristic endoscopic appearance of extensive ulceration of the mid- to distal esophageal or gastric mucosa and responsiveness to anti-CMV therapy, was compared with laboratory investigations of biopsies. Laboratory procedures consisted of both histopathological examination of the biopsy specimens and viral culture. Twenty episodes in 12 HIV-infected patients could be evaluated. Clinical diagnosis of CMV ulceration appeared to be justified in 14 of 20 episodes (70%), which were confirmed by laboratory investigations. Of the remaining six episodes, which showed partial or no response to anti-CMV therapy, laboratory investigations were negative in two episodes and discrepant in four episodes (histopathology or viral culture positive). A good response to anti-CMV therapy was more frequent in patients whose biopsies proved positive by histopathological examination and/or viral culture than in patients with negative tests (82% versus 0%), which indicates the importance of both investigations. In conclusion, laboratory diagnosis of CMV-induced upper gastrointestinal tract ulcerations supported the diagnosis and decisions on treatment of CMV-induced upper gastrointestinal tract ulcerations.

KEY WORDS: esophageal ulceration, AIDS, immunohistology, shell vial culture, anti-CMV therapy

INTRODUCTION

Infection with cytomegalovirus (CMV) is common in most HIV-infected populations, and among homosexual men with an HIV infection, seropositivity for CMV approaches 100% [Quinnan et al., 1984; Collier et al., 1987]. The prevalence of CMV *disease* in HIV-infected people is more difficult to determine. A discrepancy has been demonstrated between the postmortem presence of CMV inclusion bodies and the premortem anticipation of CMV end-organ disease [McKenzie et al., 1991]. Clinical syndromes associated with CMV end-organ disease include retinitis, gastrointestinal tract infections, colitis, encephalitis, polyradiculitis, pneumonitis and adrenalitis [Jacobson and Mills, 1988].

Since CMV reactivation and CMV end-organ disease coincides with an increasing impairment of cell-mediated immunity, it will occur more frequently as survival in advanced HIV infection improves. The availability of anti-CMV agents and the consequences of the administration of these drugs makes a proper diagnosis of CMV end-organ disease highly desirable. The role of histopathological examination and culture of the virus in order to diagnose CMV end-organ disease is still controversial and depends, among others, on the site of infection (accessibility to biopsy), detection of CMV viremia [Salmon et al., 1990] and the techniques used [Cotte et al., 1993].

We reviewed the medical records of HIV-infected patients admitted to hospital in order to determine the value of laboratory investigations (histopathology and viral culture) for the diagnosis and treatment of CMV-induced upper gastrointestinal tract ulcerations.

Accepted in publication December 14, 1995.

Address reprint requests to J.W. Dorigo-Zetsma, M.D., Laboratory of Medical Microbiology, Department of Clinical Virology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

MATERIALS AND METHODS

Patients

We reviewed the medical records of patients admitted to the clinical AIDS unit of the Academic Medical Center, Amsterdam, between September 1, 1992 and September 1, 1993. Patients were considered eligible for the study if they had ulcers in the esophagus or stomach. Episodes of upper gastrointestinal tract ulcerations that had occurred in these patients before September 1, 1992 were also included. Episodes were considered evaluable only if both histopathological and virological investigations of multiple biopsies had been undertaken and had yielded interpretable results. Excluded were episodes in which other causes of ulcerations were demonstrated, i.e., Candida infection, reflux esophagitis and peptic ulcer disease or medication. The value of laboratory investigations was determined by comparison to the clinical diagnosis of CMV ulceration: endoscopic appearance of esophageal/gastric mucosal lesions compatible with CMV ulceration, e.g., extensive ulceration of the mid- to distal esophagus, and complete response to ganciclovir and/or foscarnet.

Anti-CMV therapy was started immediately after biopsies were taken. Repeat endoscopy to judge the effect of anti-CMV therapy was performed two to three weeks after start of therapy. Response to therapy was considered to be good if complaints diminished considerably and if, on repeat endoscopy, the ulcer was re-epithelialized; it was considered to be partial if there was subjective improvement and if, on repeat endoscopy the ulcer was diminished but not completely re-epithelialized; it was considered as no response if, on repeat endoscopy, there were no signs of re-epithelialization.

Viral Culture

Specimens for CMV culture were transported in 1.5 ml transport medium (Mem-Hanks, bicarbonate 7.5%, gelatine 5%, penicillin, streptomycin, fungizone) to the viral culture laboratory and processed as quickly as possible, and within 24 hr. Biopsies were homogenized and suspended in phosphate-buffered saline (PBS), inoculated onto a monolayer of human embryonic lung fibroblasts and incubated for 0.5 hr, whereafter MEMS-medium (with 2% fetal calf serum [FCS], penicillin, streptomycin) was added. Media were changed 24 hr after inoculation and twice weekly thereafter. CMV was identified by its characteristic cytopathic effect; all cell-culture tubes were examined twice weekly for at least 6 weeks before being discarded as negative (conventional method).

At the same time, homogenized biopsy suspension was inoculated to 2 wells of a 24-well plate and processed according to the shell-vial technique [Gleaves et al., 1985]. Following 16–24 hr cocultivation, cell monolayers were fixed and stained by immunofluorescence by using a monoclonal antibody against immediate-early antigen of CMV (E13, Biosoft Lab, France) by the shell-vial technique.

For detection of CMV viremia, 10 ml EDTA blood was centrifuged, buffy-coated cells were inoculated onto cell-culture tubes (conventional method) and cocultivated with human embryonic lung fibroblasts in a 24-well plate (shell-vial technique). Results from the shell-vial technique were available within 36 hours after processing the specimen.

Histopathology

Biopsies were fixed in 10% buffered formalin (pH 7.4) and embedded in paraffin, processed, and stained routinely with hematoxylin and eosin (H and E). Four-μm-thick sections were used routinely for immunohistochemical detection of CMV, using a monoclonal antibody against immediate-early antigen of CMV (E13, Biosoft Lab) with the streptavidin method. Before incubation, the slides were deparaffinized and pretreated with pepsin 0.25% in 0.01 HCl for 10 minutes. Slides incubated with 10% normal serum before the streptavidin method were used as negative controls. Slides from known positive tissue served as positive controls. The slides were read as positive when nuclei of epithelial, endothelial or stromal cells stained brightly with the antibody, with or without the presence of nuclear inclusions in the H and E-stained slides.

RESULTS

The entry criteria were fulfilled in 27 episodes in 13 patients. Four episodes in which either no virological or histopathological examination of biopsies had been carried out, or in which these examinations were not evaluable, were excluded. Another two episodes were excluded in which the patients refused anti-CMV therapy. One episode was excluded because of a demonstrable Candida infection. Twenty episodes in 12 patients remained; all of these patients were homosexual men. Of these patients, six suffered only one episode of ulceration, four patients experienced two episodes and two patients had three episodes each. AIDS had been diagnosed at a median of 12 months (range 0–38 months) before the first episode of ulceration. The median CD4 cell count at the time of the first episode was 10/mm^3. Localization of the ulcers, laboratory investigations, and response to therapy are shown in Table I..

In 14 of 20 episodes, a good response to anti-CMV therapy was observed: 10 were successfully treated with ganciclovir alone, four received foscarnet in addition to ganciclovir. In three episodes, a partial response to ganciclovir and/or foscarnet was observed. In the remaining three episodes no response to anti-CMV therapy was observed. In these three episodes (I,J,K) a remarkable improvement or corticosteroid therapy was observed, as well as in one (L) of the three episodes in which only a partial response to antiviral therapy was observed. In two of these episodes (J and K) no evidence of CMV infection was detected, whereas in episodes (I) and (L) CMV was found in biopsies. Biopsies from ulcerations in 15 (75%) of the 20 episodes were positive for CMV by histopathological examination, whereas 13 (65%) were positive for CMV by shell vial viral culture (Table II). In 15 (75%) of the 20 episodes, histopathological examination and shell vial viral culture were concordant.

TABLE I. Localization of Upper Gastrointestinal Tract Ulcerations, CMV Laboratory Investigations and Response to Therapy in 12 HIV-Infected Patients

Patient	Localization of ulcer	Duration of AIDS (mo)[a]	CD4-count (cells/mm^3)	Biopsy: Histo-pathology	Biopsy: Viral culture[b]	Buffy coat[b]	Therapy[c]	Response	Survival (mo)[d]
A	1. esophagus	0	20	+	−/−	−/−	gcv	good	
	2. esophagus	5	nt	−	+/−	nt[e]	gcv	good	
	3. esophagus	8	10	+	+/+	+/+	gcv	good	9
B	1. esophagus	6	70	+	+/−	−/−	gcv	good	
	2. esophagus	12	<10	+	+/ne[e]	nt	gcv	partial	
	3. esophagus	15	nt	+	+/+	−/−	fosc	good	16
C	1. esophagus	12	10	+	+/+	+/+	gcv	good	3
D	1. esophagus	38	10	+	+/−	+/−	gcv	good	
	2. esophagus	48	<10	+	+/+	nt	gcv	good	9.5, alive
E	1. esophagus	24	30	+	+/−	nt	gcv	good	
	2. esophagus	26	10	−	−/−	−/−	gcv	partial	10
F	1. esophagus	16	10	+	+/−	−/−	gcv	good	
	2. esophagus	22	nt	+	+/−	−/−	gcv + fosc	good	6.5, alive
G	1. stomach	13	<10	+	+/−	nt	gcv + fosc	good	
	2. stomach	25		+	+/+	+/+	gcv	good	27
H	1. esophagus	0	<10	+	−/−	nt	gcv	good	10
I	1. esophagus	10	130	−	+/−	−/−	gcv + fosc	none	
							predn	good	5, alive
J	1. esophagus	?	130	−	−/−	−/−	gcv + fosc	none	
							predn	good	5, alive
K	1. esophagus	28	30	−	−/−	−/−	gcv	none	
							predn	good	7, alive
L	1. esophagus	0	10	+	−/−	−/−	gcv + fosc	partial	
							predn	good	6.5, alive

[a] At the time of diagnosis.
[b] Shell vial technique/conventional culture.
[c] gcv, ganciclovir; fosc, foscarnet; predn, prednisone.
[d] From the time of diagnosis of upper gastrointestinal tract ulceration up to September 1, 1993.
[e] ne, not evaluable; nt, not tested.

TABLE II. Concordance Between Histopathological Examination and Viral Culture of Biopsies and Response to Anti-CMV Therapy in 20 Episodes of Upper Gastrointestinal Tract Ulcerations

		Response to anti-CMV therapy: good	partial	none
Histopathology	+	11	1	
Virology	+			
Histopathology	+	2	1[a]	
Virology	−			
Histopathology	−	1		1[a]
Virology	+			
Histopathology	−		1	2[a]
Virology	−			

[a] Good response to steroid therapy.

Although all 20 episodes described were at first considered on macroscopic appearance to be CMV-induced, anti-CMV therapy led to complete re-epithelialization of the ulcer in only 14 patients. A good treatment response was more frequent in patients whose biopsies proved to be positive by histopathological examination and/or viral culture than in other patients (14/17 [82%] versus 0/3 [0%], $P = 0.004$, Fisher's exact test (Table II). In 14 out of the 20 episodes, culture of the buffy coats was undertaken. Four of these were positive by the shell vial viral culture. In all four of these episodes, a good response to anti-CMV therapy was found. In the 10 episodes with a negative buffy coat, five had a response to antiviral therapy (Table III).

DISCUSSION

Clinical diagnosis of CMV ulcerations, based on macroscopic appearance of the ulceration and response to anti-CMV therapy, appeared to be justified in 14 our of 20 (70%) episodes. The predictive value of a positive CMV test (i.e., histopathology and viral culture) for responsiveness to anti-CMV therapy was 92%, i.e., in 11 out of 12 episodes with a positive CMV test the response to anti-CMV therapy was good. Laboratory investigations in the three partial responders (histopathological examination and viral culture both positive, positive and negative, or both negative, respectively) were more difficult to interpret. The partial response to ganciclovir in episodes B2 and E2 might have been due to a developed resistance to ganciclovir, since those patients had been treated previously with ganciclovir. In episode L, the partial response to ganciclovir and foscarnet can be ascrived to an acquired resistant CMV strain, since this patient had not been treated previously with either drug. But partial response to anti-CMV therapy can also be the reflection of late initiation of therapy or can be explained if the ulcerations are considered not to be CMV-induced. Although both the histopathological and virological laboratory investigation was negative for CMV

TABLE III. Correlation Between Buffy Coat and Response to Anti-CMV Therapy in 14 of 20 Episodes of Upper Gastrointestinal Tract Ulcerations

		Response to anti-CMV therapy	
		Good	Partial (n = 2) or none (n = 3)
Buffy coat	+	4	0
	−	5	5

in episode E2, the partial response to anti-CMV therapy did not exclude a role for this treatment.

Difficulties in the interpretation of the laboratory investigations for CMV end-organ disease can be viewed from different angles. Inclusion bodies, detected by the histopathological examination of biopsies by routine hematoxylin and eosin staining, are an indication for active viral replication. Sinzger et al. [1993] showed recently that staining with monoclonal antibodies (Mab) against immediate-early antigen points to an early phase in the viral life cycle coupled with morphologically unaltered cells as well as cytomegalic cells. Discrepancies in the laboratory diagnoses (histopathological and virological techniques), as seen in some of the patients, can be the result of sampling errors or staining with monoclonal antibodies against immediate-early antigen, which can be the reflection of an abortive infection. Clinical use of culturing CMV for diagnosing CMV end-organ disease has been discussed previously [Salmon et al., 1990; Zurlo et al., 1993; Reiter et al., 1993]. In studies comparable to our study, Wilcox et al. [1990] did not observe any additional value for the diagnosis of CMV-induced upper gastrointestinal tract ulcerations by culturing the virus from biopsy specimen, whereas Lim et al. [1993] observed that three out of six CMV culture positive biopsy specimens would have been missed if only histopathology would have been used for detection of CMV. Although in our own study concordance between shell vial culture and histopathology was observed in 75% of the episodes, the use of both techniques is justified because of the discrepant laboratory results. The importance of carrying out both laboratory tests is stressed by the fact that good treatment response in patients whose biopsies were positive by histopathology and/or viral culture was more frequent statistically than in patients with negative tests. When studying the development of resistance to antiviral therapy, culturing of the virus is indispensable. Moreover, evidence of CMV viremia can be helpful for diagnosing CMV end-organ disease and for monitoring therapy [Salmon et al., 1990]. In our patient population all patients with CMV viremia (based on a positive CMV culture of the buffy coat) had a good response to antiviral therapy.

To date, pathogenetic mechanisms of CMV which cause disease are not fully understood [Griffiths and Grundy, 1988]. In the case of CMV retinitis or encephalitis, cell damage as a direct consequence of viral replication is thought to be the causative mechanism. On the other hand, a virus-induced immunopathological mechanism has been suggested to cause vasculitis, which can lead to ulceration of the mucosa of the gastrointestinal tract [Griffiths and Grundy, 1988; St. Onge and Bezahler, 1982].

The high CD4 cell count in two of the "steroid responders" was remarkable, which should argue against CMV end-organ disease since CMV disease usually occurs at the final stage of impaired cell-mediated immunity. Two questions arise in those cases where improvement on steroid treatment was observed, even though evidence of CMV in the biopsy specimen existed. First, was CMV the causative agent of the esophagus ulcerations, and second, if so, can a virus-induced immunopathological mechanism be treated with steroids and thus lead to the healing of the mucosal ulceration? However, based on studies with transplant patients, CMV pneumonitis is not ameliorated by steroid therapy [Reed et al., 1986]. In the other two steroid responders, evidence of positive laboratory investigations for CMV was not found either in biopsy nor in buffy coat and the diagnosis was changed into aphthous ulceration of the gastrointestinal tract mucosa; no underlying infectious agent or neoplasm was found and the patients improved remarkably on corticosteroid therapy [Bach et al., 1990; Reyes-Teran et al., 1992; Dretler and Rausher, 1989; Slomianski et al., 1992]. Pathogenesis of this type of ulcer is not completely understood, but it has been suggested that activated T-cells and, consequently, the cytokine network mediate cytotoxic effects [Reyes-Teran et al., 1992]. None of the patients treated successfully with corticosteroid therapy was viremic at the time of the diagnosis of upper gastrointestinal tract ulcerations. However, a single negative culture of a buffy coat does not exclude the possibility of a CMV-induced upper gastrointestinal tract ulceration. As is clear from Table III, some of the patients with a negative buffy coat apparently did suffer from CMV-induced ulcerations.

Little is known about the natural course of CMV-induced upper gastrointestinal tract ulcerations. CMV end-organ disease occurs at a final stage of impaired cell-mediated immunity, and survival after diagnosis of CMV-induced upper gastrointestinal tract ulceration has been described to range between 1 and 22 months [Wilcox et al., 1990; Connolly et al., 1989]. In conclusion, laboratory investigations support the clinical diagnosis of CMV ulcerations. Additional value (change in diagnosis and treatment) was obtained when laboratory investigations of ulcer biopsies were negative.

REFERENCES

Bach MC, Howell DA, Valenti AJ, Smith TJ, Winslow DL (1990): Aphthous ulceration of the gastrointestinal tract in patients with the acquired immunodeficiency syndrome. Annals of Internal Medicine 112:465–466.

Collier AC, Meyers JD, Corey L, Murphy VL, Roberts PL, Handsfield HH (1987): Cytomegalovirus infection in homosexual men: Relationship to sexual practices, antibody to human immunodeficiency virus, and cell-mediated immunity. American Journal of Medicine 82:593–601.

Connolly GM, Hawkins D, Harcourt-Webstre JN, Parsons PA, Husain OA, Gazzard BG (1989): Oesophageal symptoms, their causes, treatment and prognosis in patients with the acquired immunodeficiency syndrome. Gut 30:1033–1039.

Cotte L, Drouet E, Bissuel F, Denoyel GA, Trepo C (1993): Diagnostic

value of amplification of human cytomegalovirus DNA from biopsies from human immunodeficiency virus-infected patients. Journal of Clinical Microbiology 31:2066–2069.

Dretler RH, Rausher DB (1989): Giant esophageal ulcer healed with steroid therapy in an AIDS patient. Reviews Infectious Diseases 11:768–769.

Gleaves CA, Smith TF, Shuster EZ, Pearson GR (1985): Comparison of standard tube and shell vial cell culture techniques for the detection of CMV in clinical specimens. Journal of Clinical Microbiology 21:217–221.

Griffiths PD, Grundy JE (1988): The status of CMV as a pathogen. Epidemiology and Infection 100:1–15.

Jacobson MA, Mills J (1988): Serious cytomegalovirus disease in the acquired immunodeficiency syndrome (AIDS). Annals of Internal Medicine 108:585–594.

Lim SG, Lipman M Cl, Squire S, Pillay D, Gillespie S, Sankey EA, Dhillan AP, Johnson MA, Lee CA, Pounder RE (1993): Audit of endoscopic surveillance biopsy specimens in HIV positive patients with symptoms. Gut 34:1429–1432.

McKenzie R, Travis WD, Dolan SA, Pittaluga S, Feuerstein IM, Shelhamer J, Yarchoan R, Masur H (1991): The causes of death in patients with human immunodeficiency virus infection: A clinical and pathologic study with emphasis on the role of pulmonary diseases. Medicine (Baltimore) 70:326–743.

Quinnan GV, Masur H, Rook AH, Armstrong G, Frederik WR, Epstein J, Manichewitz JF, Macher AM, Jackson L, Ames J, Smith HA, Parker M, Pearson GR, Parillo J, Mitchell C, Strauss SE (1984): Herpes virus infections in the acquired immune deficiency syndrome. Journal of the American Medical Association 252:72–77.

Reed EC, Dandliker PS Meyers JD (1986): Treatment of cytomegalovirus pneumonia with 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl] guanine and high dose corticosteroids. Annals of Internal Medicine 105:214–215.

Reiter WM, Cimoch PJ, Keller RH. Dubé MP, Sattler FR (1993): How useful are cytomegalovirus cultures in patients with AIDS? Letter Annals of Internal Medicine 119:247–248.

Reyes-Teran G, Ramirez-Amador V, De la Rosa E, Gonzalez-Guevera M, Ponce-Leon S (1992): Major recurrent oral ulcers in AIDS: Report of three cases. Journal of Oral Pathology and Medicine; 21:409–411.

Salmon D, Lacassin F, Harzic M, Leport C, Perronne C, Bricaire F, Brun-Vezinet F, Vilde JL (1990): Predictive value of cytomegalovirus viraemia for the occurrence of CMV organ involvement in AIDS. Journal of Medical Virology 32:160–163.

Sinzger C, Plachter B, Steglein S, Jahn G (1993): Immunohistochemical detection of viral antigens in smooth muscle, stromal, and epithelial cells from acute human cytomegalovirus gastritis. Journal of Infectious Diseases 167:1427–1432.

Slomianski A, Snyder M, Goldmeier P, Fedreman M, Rivera E, Sklar M, Bloom, R (1992): Concomitant esophageal and penile ulcerations healed with steroid therapy in a patient with AIDS: Case report. Clinical Infectious Diseases 15:861–862.

St. Onge G, Bezahler GH (1982): Giant esophageal ulcer associated with cytomegalovirus. Gastroenterology 83:127–130.

Wilcox CM, Diehl DL, Cello JP, Margaretten W, Jacobson MA (1990): Cytomegalovirus esophagitis in patients with AIDS: A clinical, endoscopic and pathologic correlation. Annals of Internal Medicine 113:589–593.

Zurlo JJ, O'Neill D, Polis MA, Manischewitz J, Yarchoan R, Baseler M, Clifford Lane H, Masur H (1993): Lack of clinical utility of cytomegalovirus blood and urine cultures in patients with HIV infection. Annals of Internal Medicine 118:12–17.